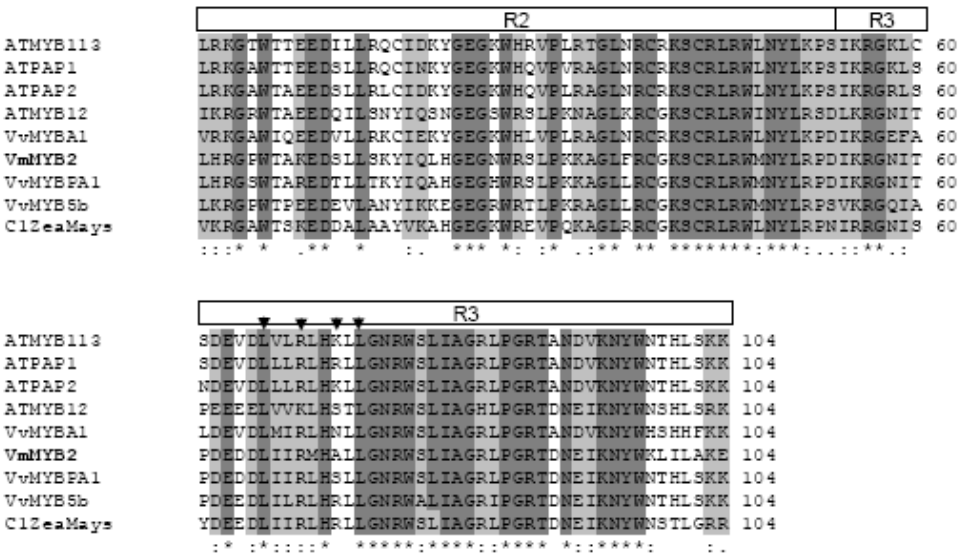
A SQUAMOSA MADS-box gene involved in the regulation of anthocyanin accumulation in bilberry fruits

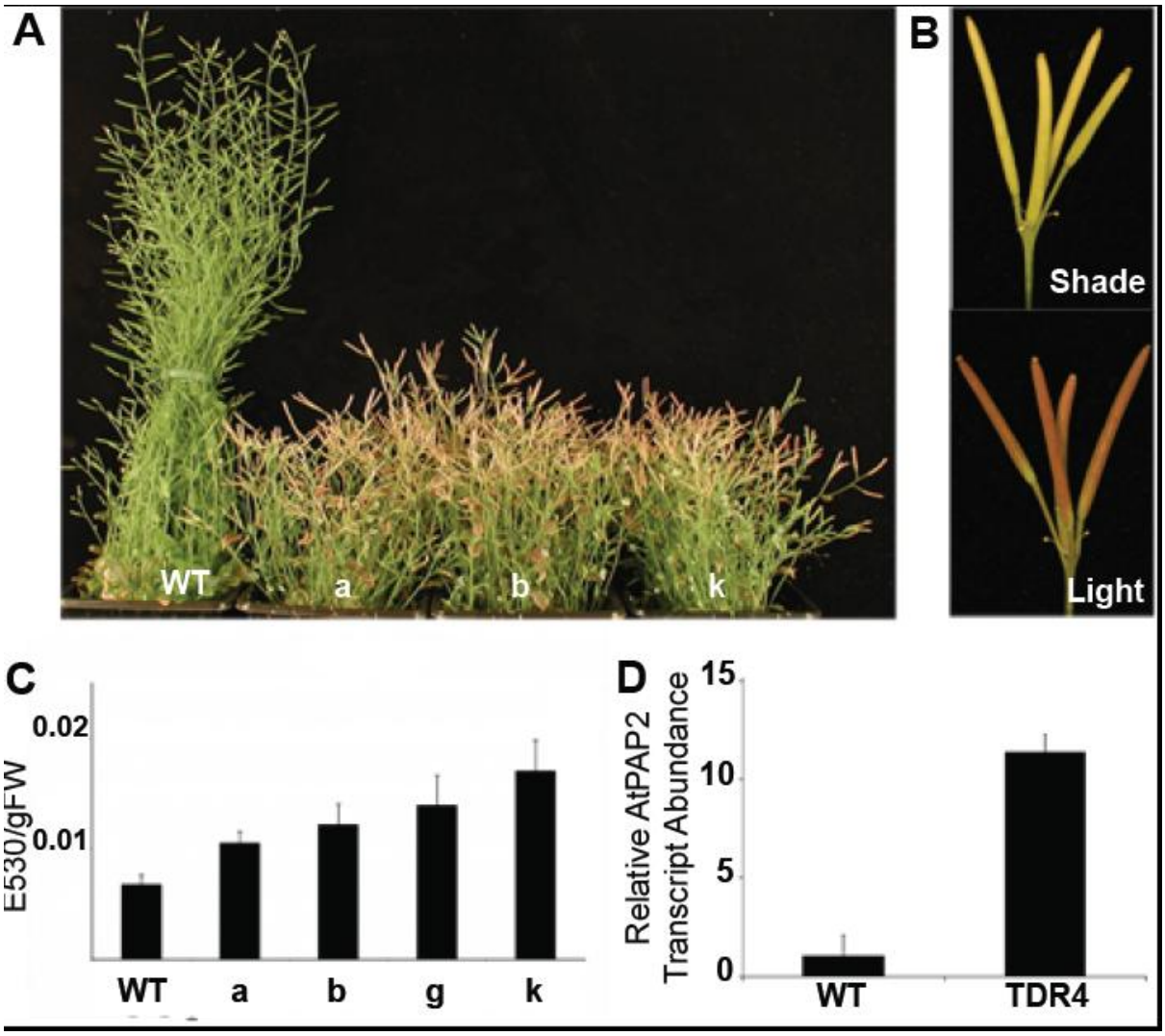
Laura Jaakola, Mervin Poole[2] , Matthew O. Jones[3], Terttu Kämäräinen-Karppinen, Janne J. Koskimäki, Anja Hohtola, Hely Häggman, Paul Fraser, Ken Manning, Graham J. King, Helen Thomson, Graham B. Seymour*

Supplementary data

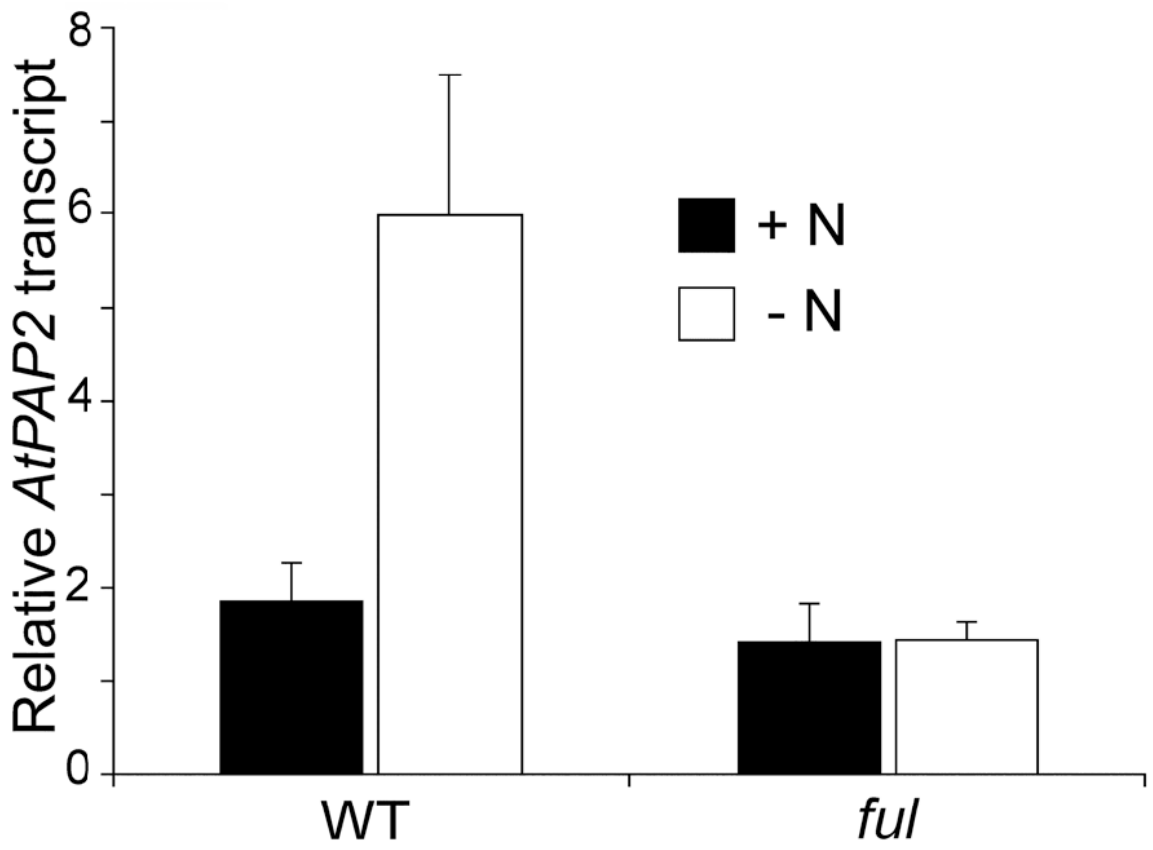


Supplementary Figure 1: Alignment of R2 and R3 DNA-binding regions of VmMYB2 with Arabidopsis PAP1, PAP2, ATMYB113 and ATMYB12, *Vitis vinifera* VvMYBA1, VvMYBPA1, VvMYB5b and Zea mays C1 anthocyanin regulator. The amino acid residues shown to be required for interaction with of the Zea mays C1 with a bHLH cofactor R are marked with arrows.

We observed that tomato *TDR4* induced anthocyanin biosynthesis when it was expressed ectopically in Arabidopsis siliques (Supplementary Fig. 2). Furthermore, in Arabidopsis the endogenous *FUL* gene, which is strongly related to *TDR4* and *VmTDR4* by sequence homology, is necessary for the expression of the MYB transcription factor *PAP2* under condition of nitrogen starvation (Lea et al, (2007) (Supplementary Fig. 3).



Supplementary Fig.2. Effects of ectopic expression of tomato *TDR4* in Arabidopsis. (A) *TDR4* transgenic lines (a,b,g,k) display reduced stature in comparison to wild type (WT), k not shown. (B) *TDR4* siliques revealing differential accumulation of red pigmentation in light. (C) Quantification of anthocyanin accumulation in Arabidopsis siliques collected from lines a, b, g and k. (D) Quantification of *AtPAP2* transcript abundance in WT and *TDR4* lines (mean of a, g and k, error bars, SEM, n=3).



Supplementary Fig. 3. Determination of *AtPAP2* transcript abundance by quantitative PCR of cDNA in WT and *fruitfull* (*ful*) mutant leaves in response to growth with and without nitrogen (N, error bars, SEM, n=3).

Materials and Methods

Arabidopsis Col-0 plants were transformed by floral dip with the *TDR4* coding sequence under the control of the cauliflower mosaic *virus* 35S promoter. Transformed plants were selected by germination on compost containing the herbicide Basta. The mRNA coding region for the *TDR4* protein sequence was amplified from *Solanum lycopersicum* cultivar "Ailsa Craig" pericarp total cDNA using primers, LEB1TDR4F (5'-AAAAAGCAGGCTAAAAAATGGGAAGAGGAAGAGTC-3') and LEB2TDR4R (5'-AGAAAGCTGGGTATACCTTTTAATTATTAAG-3') were designed to include the methionine and translation stop codons of *TDR4* (SGN, Unigene sequence U144041). The primers were gateway adapted to allow cloning *via* pDONR221 (Invitrogen) into the pB7WG2 plant expression vector by BP and LR recombination reactions (Invitrogen). The T-DNA generated was designed to transcribe *TDR4* under the control of the CaMV 35S promoter away from right border.

Anthocyanin concentrations in silique tissues were determined by spectrophotometric quantification in acidic methanol as previously described (Rabino and Mancinelli, 1986).

References

Lea U, Slimestad R, Smedvig P, Lillo C (2007) Nitrogen deficiency enhances expression of specific MYB and bHLH transcription factors and accumulation of end products in the flavonoid pathway. Planta **225:** 1245-1253

Rabino I, Mancinelli AL (1986) Light, temperature and anthocyanin production. Plant Physiology 81, 922-924.